

NON-SYMBIOTIC GERMINATION OF ORCHIDS

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The propagation of orchids from seeds is a problem which has attracted the attention of many investigators. That orchids could be grown in the greenhouse from seeds was not considered extraordinary; orchid growers in Europe and in this country were successful in this line of work. Naturally scientists took up the study of the conditions of germination. In the roots of the orchid plants symbiotic fungi had been detected, and the first investigators of this problem, Bernard ('09) and Burgeff ('09) undertook experiments with the symbiotic fungus. Since they had no success without the fungus, the presence of the fungus was considered to be a *conditio sine qua non*. In 1922 Knudson published the results of his successful experiments on the non-symbiotic germination of orchids, and a number of other investigators have worked on the same subject, in the majority of cases successfully. It is a well-known fact that a greater knowledge of the conditions for growing orchids is of particular interest not only to those working on the subject for commercial purposes, but also to those engaged in scientific investigations.

From Knudson's ('25, '27) experiments we learn that the metabolic processes of the fungus bring about chemical changes in the medium which make it more assimilable. Chemical compounds indigestible for the seedlings are broken up or transformed into ones which can be easily used as food by the embryo. Another favorable influence of the fungus is the gradual acidification of the medium, according to Burgeff, Knudson, and Clement. However, experiments showed that it need not be necessarily the special orchid fungus. Other fungi may produce the same effect. Every one dealing with the germination of orchid seeds will have made the same discovery. From the experiments of Knudson and other investigators, such as Constantin and Magrou ('22), Ramsbottom ('22), and Clement ('24, '26, '29), our present knowledge of the requirements for successful germination of

orchid seeds can be summed up as follows: a suitable solution of mineral salts, a satisfactory sugar supply, and a favorable hydrogen-ion concentration. These are conditions which can be obtained by a nutrient solution, the composition of which has been determined by previous investigations. This has been proved in a great number of experiments on seeds from different species of orchids, and there is no doubt that further investigations will show that the theory holds for all species of orchids. If we take into consideration these three main requirements, mentioned above, and determine, by changing the composition of the medium, the best conditions of germination, we will be able to give a list of the most favorable nutrient solutions for germinating seeds of each species of orchids.

In preliminary experiments nutrient solutions containing only sugars, three-times distilled water, and agar (Merck's Reagent) which had been washed for three weeks in thrice-distilled water and carefully dried in a desiccator over concentrated sulphuric acid, were used. Maltose, glucose, levulose, and saccharose (Merck Reagents) were used as sugar supplies. The best germination and growth of *Cattleya* seeds were recorded on nutrient media with maltose and at pH values between 4.8 and 5.4. The seedlings developed two leaves within five months and appeared healthy. Transplanted to full nutrient solutions with a suitable sugar supply development and growth continued as in seedlings germinated the usual way. The results prove the importance of carbohydrates in the germination of orchid seeds. For this reason the experiments with solid media were carried out and the results are reported here.

It is hoped that the experiments reported in this paper may be a contribution to the subject.

METHODS

As culture vessels Erlenmeyer-flasks of "Pyrex" glass of 250-cc. capacity were used. One hundred cc. of nutrient agar was poured into each flask, the neck of the flasks closed with a cotton plug, and the flasks sterilized in an autoclave for 30 minutes under a pressure of 10 lbs. Then the medium was allowed to cool in a slanting position. The medium was so

adjusted before sterilization that when the process was concluded the desired pH resulted, as was shown by tests. All seeds were sterilized in chlorinated lime solution for 30 minutes, according to the procedure recommended by Wilson ('15). During this time the solution was shaken frequently to moisten all seeds. The sterilizing solution was then poured out and sterilized distilled water added under sterile conditions, and in this the seeds remained until they were planted on the agar slant with the aid of a sterilized platinum loop. After the seeds were planted, the cotton plug and the neck of the culture flask were covered with a double layer of waxed paper fastened with a rubber band to prevent too rapid an evaporation of the water, especially in extremely hot weather. Conditions in the greenhouse made it necessary to add sterile water to the cultures from time to time, usually after each sixth week. Although this was done with all possible precautions contamination, which often destroyed as many as 10 per cent of the experiments, could not be avoided. Therefore all experiments were carried out in triplicate.

It might be mentioned here that a number of different nutrient solutions was tried out with regard to the effect on germination of orchid seeds. The nutrient solutions used were those employed by Artari, Molisch, von der Crone, Nobbe, Pfeffer, Sachs, and three different variations of Knop's solution. Although a definite decision cannot be made at present, the best growth and development were recorded in the nutrient solutions employed by Knop and Sachs. However, the growth of seedlings in these solutions was only 50 per cent of that obtained during the same period in the solutions of Burgeff, Pfeffer, and Knudson's solution "D." Since the nutrient solution "B" employed by Knudson ('22) and the one developed by the writer, here called solution "L," proved most satisfactory, they alone are considered in this paper.

Solution B		Solution L	
Ca(NO ₃) ₂	1.00 gm.	CaCl ₂	1.00 gm.
K ₂ HPO ₄	0.25 gm.	KH ₂ PO ₄	1.00 gm.
MgSO ₄ . 7H ₂ O	0.25 gm.	MgSO ₄ . 7H ₂ O	1.00 gm.
Fe ₂ (PO ₄) ₂ . 4H ₂ O	0.25 gm.	Fe ₃ (PO ₄) ₂ . 8H ₂ O	0.33 gm.
(NH ₄) ₂ . SO ₄	0.50 gm.	(NH ₄) ₂ .CO ₃ .H ₂ O	0.50 gm.
Sugar	20.00 gm.	NH ₄ .NO ₃	0.50 gm.
Distilled water	1000 cc.	Sugar	20.00 gm.
		Distilled water	1000 cc.

In general 1.75 per cent agar was added. Preliminary experiments had shown that orchid seeds planted on medium L finally grew as well as in Burgeff's solution. Acid was added in a quantity necessary to bring the pH concentration to the point desired. Of course, in solutions with pH values below 4.8 the percentage of the agar had to be raised in order to render the medium solid enough for the purpose designated. After planting, the culture flasks were placed in the greenhouse at a temperature between 20° and 30° C. and kept covered with cheese-cloth.

It was possible to make measurements with an ocular micrometer attached to the microscope, but only of those seedlings growing close to the wall of the flask. However, since the number of seedlings in one flask never exceeded 300, the seedlings in the same stage of growth and of the same size could be examined with relative ease and the percentage of the different sizes determined.

The seeds used in these experiments were those from *Cattleya* hybrids grown at the Missouri Botanical Garden, and were over one year old. The parents are as follows:

No. 18. *Cattleya Trianae* × *Laelio-Cattleya luminosa aurea*.

No. 21. *Cattleya Trianae* × *Cattleya* "Princess Royal" *alba*.

No. 27. *Cattleya Trianae* (*light*) × *Cattleya O'Brieniana*.

In general, the terms used in describing the stages of development are those employed by Knudson ('22); the expression "advanced spherule stage" used in this paper means the period of production of leaves before the formation of the first root.

EXPERIMENTS

Experiments were carried on with solution B and the writer's solution L, to which had been added levulose, glucose, sucrose, or maltose, as the source of sugar. The seeds were planted on July 19, 1927, and the results here reported recorded on December 29, 1927.

The measurements were taken in the manner described above; for this reason the figures given are only approximate, but are under- rather than over-estimated. The numbers given in the column "succession in superiority" express the following three stages, the average measurements of the embryos being used: (1) average diameter above 1000 μ , (2) average diameter from 500 μ to 1000 μ , and (3) average diameter below 500 μ .

The results of these experiments, recorded in table I, prove that the best growth was obtained in nutrient solutions with maltose as the sugar supply.

TABLE I

CATTLEYA SEEDS PLANTED ON JULY 19, 1927; GROWTH RECORDED ON DECEMBER 29, 1927

No. of seed	Nutrient solution	Diameter of embryo in microns			State of growth	Succession in superiority
		Min.	Max.	Aver.		
18 21 27	Plain B	90 85 100	140 180 200	115 123 150	Small spherule stage; very light green; seedlings apparently dying.	3* 3 3
18 21 27	Solution B + 2% levulose	460 490 500	1200 1500 1400	830 995 950	Seedlings forming leaves; green; healthy.	2 2 2
18 21 27	Solution B + 2% glucose	460 480 450	1100 1200 1300	785 840 875	Seedlings green; forming leaves; well developed.	2 2 2
18 21 27	Solution B + 2% sucrose	450 460 500	1000 900 1100	725 680 800	Seedlings well developed; green; forming leaves.	2 2 2
18 21 27	Solution B + 2% maltose	850 960 1000	1000 1100 1200	925 1030 1100	Seedlings very well developed; green; slender; healthy; forming leaves.	2 1 1
18 21 27	Plain L	100 90 100	180 200 300	140 145 200	Small spherule stage; very light green; leaf point marked.	3 3 3
18 21 27	Solution L + 2% levulose	520 680 700	1350 1400 1500	935 1040 1100	Seedlings well developed; green; healthy; forming leaves.	2 1 1
18 21 27	Solution L + 2% glucose	550 670 540	1150 1350 1450	850 1010 995	Seedlings well developed; green; forming leaves.	2 1 2
18 21 27	Solution L + 2% sucrose	490 630 480	1200 1400 1500	845 1015 990	Seedlings well developed; green; forming leaves.	2 1 2
18 21 27	Solution L + 2% maltose	890 860 1000	1400 1500 2000	1145 1180 1500	Seedlings very well developed; dark green; very well developed protocorms; all forming leaves.	1 1 1

* The numbers in this column denote the following stages: 1, average diameter above 1000 microns; 2, average diameter from 500 to 1000 microns; and 3, average diameter below 500 microns.

SOLUTION B

Maltose.—The best growth was recorded in solutions which had been supplied with maltose. Ninety per cent of the embryos were far advanced in growth, having protocorms from 1 to 1.2 mm. in diameter; each had developed two leaves (3 to 6 mm. in length). Ten per cent of the seedlings had already produced one leaf (2 to 4 mm. in length), and a second one half as long as the first was forming. All seedlings had a great number of fine root hairs and appeared healthy. The internodes and petioles were elongated and showed only a very pale green color, this condition resembling the etiolation usually ascribed to lack of nitrogen. However, development of the seedlings was otherwise apparently normal, and the leaves were vigorous, as shown by their large size. The etiolated cases in which growth was retarded may be considered as individual variations (seedlings which because of some internal factors grew more slowly). The pH concentration in these cultures was between 5.2 and 5.4, certainly sufficient to secure normal growth, as preliminary experiments had shown. Seedlings grown on media with a pH concentration above 5.6 showed very slow growth and chlorosis. They were, at the time when the experiments were recorded, in the advanced spherule stage, but were only very light yellowish with a light green spot marking the place of the leaf point. In nutrient solutions with a pH concentration above 6.0 the growth was as poor as in the plain solution without any sugar supply.

Sucrose.—All seedlings were in the advanced spherule stage, about 60 per cent of them having already formed the first leaf (0.5 to 0.8 mm. in length) and the second one just started. The rest of the seedlings were forming the first leaf. All were healthy, but the color of the leaves was not as dark as in those seedlings grown on the maltose medium. The pH concentration of these solutions with sucrose was 5.2. (On the more alkaline media the growth and development of the embryos rapidly decreased, so that at a concentration of 5.6 the seedlings showed severe chlorosis). All were in the advanced spherule stage, but only 9 per cent had formed one leaf measuring 0.3 to 0.5 mm. in length, and the remaining 91 per cent had just reached the advanced spherule stage with the leaf point barely marked. All the embryos were

very light green, except those grown at 5.2, which were almost white, only the leaf point, or in the advanced stage of growth, the leaves, showing a very light green color. Two to three weeks after these experiments were recorded even this very light green color had almost faded away, and the seedlings were apparently dying, being unable to produce chlorophyll and to carry on assimilation.

Glucose.—The development of seedlings on this medium was fairly good. They had well-developed light green protocorms; 22 per cent had already produced two leaves (1 to 1.5 mm. in length); 78 per cent were just forming the first two leaves (0.5 to 1.2 mm. in length), the second leaf one-half the length of the first. All leaves were light green, and all seedlings which had attained full growth of both leaves had formed root-hairs. The pH concentration of the nutrient medium was 5.0. In experiments with higher pH values, likewise in the experiments with maltose and sucrose, a decrease in growth and life processes has been observed. At a pH concentration above 5.6 the development was noticeably retarded; the first indication of chlorosis appeared; the embryos reached the spherule stage and even pointed the first leaf, but were not able to continue in growth. In pH concentrations of about 5.8 and higher the seedlings swelled, but the embryos remained small and showed only very light yellowish green color.

Levulose.—The development of the seedlings on this medium was very satisfactory. The seedlings were dark green, the protocorms very well developed; about 10 per cent had already formed two dark green leaves (2 to 3 mm. in length); 65 per cent had well-developed protocorms and two dark green leaves, the second one-half the length of the first. Twenty-five per cent had not progressed as far in growth and were just forming the first leaf. The effect of the pH concentration was observed to be the same as in the media mentioned above. Of the remaining 25 per cent almost all were forming two dark leaves, the protocorms being green and well developed. Only very few had not progressed as far in growth and were just forming the first leaf.

In one of the cultures made from seed No. 27 about 38 per cent of the seedlings had died in the very first spherule stage; they had

swelled but turned brown before the leaf had been pointed; 7 per cent had passed the advanced spherule stage, had green protocorms and two dark leaves (2 to 3 mm. in length), and appeared very healthy. The failure of those 38 per cent to germinate could be explained only by the weak constitution of the seed, the nutrient medium having been the same as in the other nutrient solutions. This is mentioned merely to make a complete report on the experiments.

Plain nutrient medium.—On this medium without any sugar supply the embryos grew to the small spherule stage, the protocorms being about 90 to 150 μ in diameter and showing a whitish green color with a very light green spot, the leaf point. Apparently they were dying.

SOLUTION L

Maltose.—As in solution B, in solution L, the best growth of the seedlings was registered with maltose. The embryos were very well developed, with large and dark green protocorms (1 to 2 mm. in length) and dark green leaves. Eighty per cent of the seedlings had already formed the first leaf (2 to 3 mm. in length), the second one being half the length of the first. Ten per cent had passed through the small spherule stage and the first leaf was forming. Ten per cent had two leaves (2 to 5 mm. in length) and were forming the third one. The seedlings on this nutrient medium had the largest protocorms of any in these experiments; the protocorms were green, the leaves dark green, and the seedlings with developed leaves had formed root-hairs. All seedlings appeared normal, there being no unusual elongation of the internodes or the petioles, even though they were very crowded. It seems to be necessary to mention this fact, because one might suspect that in solution B with maltose the crowded condition of the seedlings might have caused the kind of etiolation mentioned above. The seedlings were in the best state of growth and health. The pH concentration of this nutrient medium was about 5.2. Another fact which ought to be brought out is that some of the seedlings with developed leaves showed the first signs of root formation.

Sucrose.—The seedlings on this medium were well developed with green protocorms and leaves somewhat darker, but not as

dark as in the cultures with maltose. Eighty per cent of the embryos had already formed one leaf (0.5 to 1 mm. in length) and were forming the second one about one-third the length of the fully developed leaf. Fifteen per cent were forming the first leaf (half of the full length), and 5 per cent were in the advanced spherule stage, just forming the first leaf. All seedlings appeared healthy, and those with developed leaves had formed root-hairs. The pH concentration was about 5.2.

Glucose.—The seedlings grown on glucose medium were fairly well developed and green with a well-developed protocorm. Sixty-eight per cent had two leaves (1 to 1.5 mm. in length); 32 per cent were forming the first leaf which was about 0.3 to 0.8 mm. in length. The embryos with developed leaves were forming root-hairs. The seedlings on glucose medium developed somewhat differently from those grown on a maltose or levulose medium. They grew more slowly and were light green, the leaves or the leaf points being dark green. The pH concentration was 5.2. On media with a pH value of 5.6 and above, decided chlorosis was noticed, and at a pH value of about 6.0 the growth equalled that on plain nutrient medium without any sugar supply.

Levulose.—Twelve per cent of the seedlings were dark green. The protocorms were very well developed, and compared to those on the other media were second largest in diameter. They had two developed leaves (1.5 to 2 mm. in length) and were forming root-hairs. Seventy per cent of the seedlings were dark green with one developed leaf (1 to 1.5 mm.), and 18 per cent were in the advanced spherule stage, just beginning to form the first leaf. Although the protocorms and leaves were well developed the growth was apparently slower than on the other media at the same pH concentration. The most favorable hydrogen-ion concentration was about 5.2.

Plain nutrient medium.—On this medium without any sugar supply the seedlings had reached the small spherule stage, the protocorms measuring about 90 to 200 μ in diameter. They had attained the same stage of growth in 5 months as those grown on sugar media in 4 to 6 weeks. The protocorms were very light green and showed a darker spot, the leaf point, and some of the seedlings had started to form a leaf (20 to 50 μ in length). Ap-

parently, however, they were not capable of carrying on assimilation. One month after these records were taken (January 29, 1928) the seedlings were still alive.

DISCUSSION

Summarizing these results in a synoptical table we have the following data regarding the development on the different media.

TABLE II

	Solution B	Solution L
Plain	Very weak growth; seedlings apparently dying.	Weak growth; leaf-point weakly marked; continuation of growth doubtful.
+ levulose	10% with two developed leaves; 65% with one developed leaf, forming the second one; root hairs; 25% forming the first leaf. All apparently healthy.	12% with two developed leaves and root-hairs; 70% with one developed leaf; 18% in the advanced spherule stage, forming the first leaf.
+ glucose	22% with two developed leaves and root-hairs; 78% with one leaf, forming the second. Healthy.	68% with two developed leaves and root-hairs; 32% forming the first leaf. Healthy.
+ sucrose	60% with one developed leaf, the second forming; 40% forming the first leaf.	80% with one developed leaf, the second forming; root-hairs; 15% forming the first leaf; 5% in the advanced spherule stage.
+ maltose	90% with two fully developed leaves and root-hairs; 10% with one fully developed leaf, and one half-developed; root-hairs. Healthy.	10% with two fully developed leaves, forming the third, also roots; 80% with one fully developed and one half-developed leaf and root-hairs; 10% forming first leaf.

The practically negative results obtained on plain nutrient media without any sugar supply are in agreement with those of other investigators who dealt with the same subject. Although the seedlings in these solutions were still living when the data were taken, there is no doubt that they would gradually have died. This fact was proved also in a number of preliminary experiments performed in this laboratory. The conclusion that orchid seedlings grown under aseptic conditions on sugar-free media are not capable of germination is therefore highly plausible. It might be emphasized that a slight difference in the development in solution L from that in solution B was noticed, as is indicated in

the tables. While in solution B the embryos had reached only the small spherule stage and the leaf point was not even marked, those in solution L had passed through this stage and had a leaf point, although very weakly marked; some few of them had even tried to form a leaf, but apparently were unable to continue growing.

The best growth occurred on media supplied with maltose, especially in solution L. The seedlings grown on medium B were very well developed and healthy in appearance, but the protocorms were slender, the leaves narrow and lying at full length. The diameters of the protocorms in solution B were as much as 36 per cent shorter than of those grown in solution L. Referring to Knudson's experiments on symbiosis with different fungi on media supplied with starch, it is very probable that the favorable effect of certain fungi upon germination and development of orchid seeds might be ascribed to the fact that those fungi in question, by enzymatic processes, transform the starch to sugars, which are better digested by the orchid embryos, and that their metabolic processes render the medium more acid, thus favoring enzyme action. A similar explanation could be assumed in cases where a favorable effect of bacteria and yeasts had been reported. Knudson says: "When starch is provided as the organic matter in the culture media, there is no germination unless the fungus is supplied." The facts that no germination took place on starch-media not inoculated with a fungus, as reported by Knudson, and that the starch remained untouched, show distinctly that orchid seeds do not possess enzymes to transform the starch to sugar, and that they therefore are unable to use this food supply. Burgeff ('09) reports that the best growth of the symbiotic fungi was recorded on media containing agar, rain-water, and a small supply of starch. He also points out that for those fungi maltose was a better source of sugar than saccharose. From the fact that in starch hydrolysis maltose is the final product which can be transformed to glucose, also from the results obtained in the experiments reported in this paper, and finally, from the evidence that glucose is not an especially favorable sugar supply for orchid embryos, we may conclude that maltose is probably one of the best-suited foods for orchid seedlings. The probability is that

the hydrolysis of maltose to glucose furnishes energy to the growing plant. Additional credence is given this supposition by Bernard's ('09) report in which he states that the starch is transformed to sugar when the symbiotic fungus enters the cells of the germinating orchid seeds.

There is no doubt that the orchid seed contains a supply of sugar, although in very small amounts. In many tests made on seeds of *Cattleya*, *Laelio-Cattleya*, *Brasso-Cattleya*, etc., the author has always found reducing sugars. Burgeff (*l. c.*, p. 70-71) mentions that a yellow coloring matter goes into solution if orchid seeds are allowed to stand for several days in water; he supposes that this undetermined matter, which reduces Fehling's solution and therefore has the qualities of a sugar, attracts the symbiotic fungus. This undetermined reducing sugar might be considered the only source of carbohydrate for the germinating seed. If it is used up and no other suitable carbohydrate is available the embryo is doomed to die.

If we suppose the protocorms to be spheres and calculate the volume of those spheres from the average-measurements given in table I, we will find a difference in volume of as much as 2.5 times. However, the smaller the volume the less is the assimilation. This difference in the ratio of assimilation indisputably has an influence upon the growth and development of the seedlings. It is a question whether the constitution of solution L causes this difference in development. Certainly the vigorous growth of the protocorms indicates a healthy development, since the nutrient material obtained from the nutrient solution is evidently not only sufficient to maintain growth, but there is undoubtedly a surplus of material which is stored in the tissue of the protocorm.

Also the pH concentration of the medium is of great importance, a fact which was brought into prominence by Knudson and proved by Clement. As has been mentioned above, in nutrient solutions with pH values of 5.2 and below the growth takes place in a normal fashion; above 5.2 growth is markedly retarded and signs of chlorosis appear. Hydrogen-ion concentrations of about 6.0 and above are very unfavorable for growth. Usually the seeds do not germinate at all, and if they do chlorosis takes place and the seeds die after they have reached the small spherule

stage. The favorable effect of a symbiotic fungus is explained by the additional fact that the fungus produces acid, thus changing the pH concentration of the less favorable medium to one more favorable, a fact also pointed out by Knudson, as mentioned above. This indicates that when the seed is planted under aseptic conditions on a maltose medium, for example, enzymes are present in small quantities and these in turn are stimulated or retarded in their activity by acid or basic conditions.

Since hydrochloric acid was used in order to adjust the pH concentration, the amount of chloride is higher in solution L than in solution B, which contains no chlorides in the original composition. Preliminary experiments have demonstrated that the effect of chlorine on growth is not of importance, and the effect on germination is problematical; likewise it is doubtful whether the treatment of seeds before planting (with chlorinated lime) has a stimulating effect upon germination. Seeds taken under aseptic conditions from a seed pod and planted without sterilization directly on the medium germinated as well as those treated before planting with the sterilizing solution in question. This, however, does not exclude the possibility of stimulating seed germination by *hypotonic* chloride solutions when Popoff's ('25) method is employed.

A comparative examination of the two nutrient solutions shows that there is, theoretically, 5 times more potassium in solution L; further 4 times as much phosphate, and about 16 per cent more nitrogen. At first it would seem to be impossible to apply a nutrient solution of such composition, but we must take into account the fact that a relatively large amount of salt is precipitated when the solution is subjected to sterilization and to titration with either acids or alkalies. That precipitate consists, as far as could be determined qualitatively in default of an equipment for quantitative analyses, of C, K, P, and Fe, and we may suppose that the difference is not as striking as it appeared from the first. We assume that the amount of nitrogen was increased slightly, the amount of both potassium and phosphorus, many times. What is the effect upon germination and growth? According to the records summarized in the tables the development in solution L was, in general, 5 to 10 per cent better than in solution B. The

seedlings appeared darker in color and had progressed relatively further in development. This effect might be ascribed to the larger dose of phosphorus and potassium. Maiwald ('23) and Schertz ('29) found, in experiments on potato and cotton plants, a correlation between phosphorus, potassium, and nitrogen and the formation of chloroplast pigments. Nitrogen increased the amount of pigments, large amounts of phosphorus produced more chloroplasts than potassium and less than nitrogen, large amounts of potassium evidently suppressed pigment formation. Stoklasa ('16) has proved that the potassium ion plays an important part in the processes of metabolism. Clements' ('28) studies on the nutrition of pea seeds indicate that KH_2PO_4 has a very marked influence on nitrogen assimilation, and also that a satisfactory balance between nitrogen and carbohydrates is of importance. The results of the experiments in solution L can be interpreted as the effect of large doses of potassium on the synthesis of proteins (Weever, '11) and the assimilation of nitrogen, an effect connected with an increase in assimilation of carbohydrates. It must be borne in mind, however, that assimilation is also dependent on the nature of the sugars and the presence of proper enzymes in the organism. The activity of enzymes, in turn, appears to be dependent to some extent on the pH concentration of the medium.

SUMMARY

1. Of the sugars used in these experiments maltose was found to be most favorable for the germination and growth of *Cattleya* seeds. According to their effect upon growth, the other sugars rank as follows: levulose, glucose, and saccharose.

2. The hydrogen-ion concentration is of great importance in germination and development of *Cattleya* seeds, much better growth occurring in solutions with a pH value between 4.8 and 5.2.

3. On media with a pH value at or above 5.6 germination and growth are retarded and in most instances chlorosis takes place. With a pH value of 6.0 and higher there is extreme chlorosis and the seedlings are unable to germinate.

4. On glucose and sucrose, the least favorable carbohydrate sources, the seedlings develop more slowly and chlorophyll is formed less rapidly, even at the most favorable pH concentration.

5. A nutrient solution, solidified with agar, of a composition different from Knudson's solution B was tried. The growth and development on this medium are better than on solution B.

6. Factors involved in this problem are discussed.

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EXPLANATION OF PLATE

PLATE 42

- Fig. 1. *Cattleya* seedlings No. 27 grown on medium L + 2 per cent maltose. $\times \frac{9}{16}$.
- Fig. 2. *Cattleya* seedlings No. 27 grown on medium B + 2 per cent maltose. $\times \frac{9}{16}$.
- Fig. 3. *Cattleya* seedlings No. 21 grown on medium L + 2 per cent sucrose. $\times \frac{9}{16}$.
- Fig. 4. *Cattleya* seedlings No. 21 grown on medium B + 2 per cent sucrose. $\times \frac{9}{16}$.
- Fig. 5. *Cattleya* seedlings No. 27 grown on medium L + 2 per cent maltose, showing chlorosis as consequence of a pH concentration of about 5.8. $\times \frac{1}{2}$.
- Fig. 6. *Cattleya* seedlings No. 27 grown on medium L + 2 per cent sucrose; the first two seedlings on the left side are shown growing on the top of other seedlings. Natural size.